



## Other Regents for Cell Immortalization

CAT#	Product Name	Amounts
<a href="#">LVP1134-RB</a>	EBNA1 (RFP-Bsd) Lentivirus	200ul,  (1 x 10 <sup>7</sup> IFU/mL) in DMEM medium containing 10% FBS / 10 x (60 ug/m) polybrene
<a href="#">LVP1135-RB</a>	EBNA2 (RFP-Bsd) Lentivirus	
<a href="#">LVP1136-RB</a>	HPV16-E6 (RFP-Bsd) Lentivirus	
<a href="#">LVP1137-RB</a>	E1A (RFP-Bsd) Lentivirus	
<a href="#">LVP1138-RB</a>	HOXA9 (RFP-Bsd) Lentivirus	
<a href="#">LVP1139-RB</a>	KRas_G12V (RFP-Bsd) Lentivirus	
<a href="#">LVP1140-RB</a>	CDK4 (RFP-Bsd) Lentivirus	
<a href="#">LVP1141-RB</a>	cMyc (RFP-Bsd) Lentivirus	
<a href="#">LVP1134-GP</a>	EBNA1 (GFP-Puro) Lentivirus	
<a href="#">LVP1135-GP</a>	EBNA2 (GFP-Puro) Lentivirus	
<a href="#">LVP1136-GP</a>	HPV16-E6 (GFP-Puro) Lentivirus	
<a href="#">LVP1137-GP</a>	E1A (GFP-Puro) Lentivirus	
<a href="#">LVP1138-GP</a>	HOXA9 (GFP-Puro) Lentivirus	
<a href="#">LVP1139-GP</a>	KRas_G12V (GFP-Puro) Lentivirus	
<a href="#">LVP1140-GP</a>	CDK4 (GFP-Puro) Lentivirus	
<a href="#">LVP1141-GP</a>	cMyc (GFP-Puro) Lentivirus	200ul,  (1 x 10 <sup>8</sup> IFU/mL) in PBS solution
<a href="#">LVP1134-RB-PBS</a>	EBNA1 (RFP-Bsd) Lentivirus in <b>PBS</b>	
<a href="#">LVP1135-RB-PBS</a>	EBNA2 (RFP-Bsd) Lentivirus in <b>PBS</b>	
<a href="#">LVP1136-RB-PBS</a>	HPV16-E6 (RFP-Bsd) Lentivirus in <b>PBS</b>	
<a href="#">LVP1137-RB-PBS</a>	E1A (RFP-Bsd) Lentivirus in <b>PBS</b>	
<a href="#">LVP1138-RB-PBS</a>	HOXA9 (RFP-Bsd) Lentivirus in <b>PBS</b>	
<a href="#">LVP1139-RB-PBS</a>	KRas_G12V (RFP-Bsd) Lentivirus in <b>PBS</b>	
<a href="#">LVP1140-RB-PBS</a>	CDK4 (RFP-Bsd) Lentivirus in <b>PBS</b>	
<a href="#">LVP1141-RB-PBS</a>	cMyc (RFP-Bsd) Lentivirus in <b>PBS</b>	
<a href="#">LVP1134-GP-PBS</a>	EBNA1 (GFP-Puro) Lentivirus in <b>PBS</b>	
<a href="#">LVP1135-GP-PBS</a>	EBNA2 (GFP-Puro) Lentivirus in <b>PBS</b>	
<a href="#">LVP1136-GP-PBS</a>	HPV16-E6 (GFP-Puro) Lentivirus in <b>PBS</b>	
<a href="#">LVP1137-GP-PBS</a>	E1A (GFP-Puro) Lentivirus in <b>PBS</b>	
<a href="#">LVP1138-GP-PBS</a>	HOXA9 (GFP-Puro) Lentivirus in <b>PBS</b>	
<a href="#">LVP1139-GP-PBS</a>	KRas_G12V (GFP-Puro) Lentivirus in <b>PBS</b>	
<a href="#">LVP1140-GP-PBS</a>	CDK4 (GFP-Puro) Lentivirus in <b>PBS</b>	
<a href="#">LVP1141-GP-PBS</a>	cMyc (GFP-Puro) Lentivirus in <b>PBS</b>	

**Storage:** <-70 °C, avoid repeat freeze/thaw cycles, stable for > 6 months.

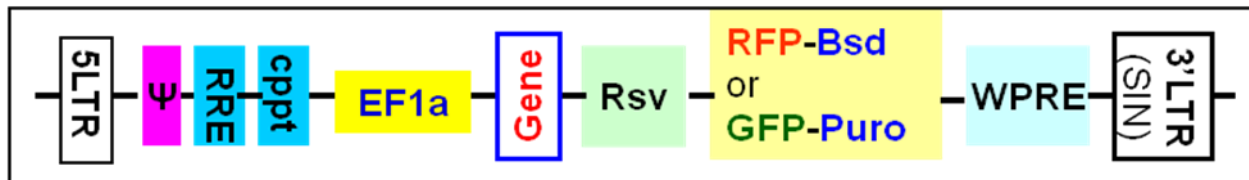


## Product Description:

Normal cells will die after a few rounds of proliferation because of cellular senescence. There are a few methods to turn a primary cell to immortal so that the cells can undergo infinite cell divisions or large rounds of doubling in culture medium (Cell immortalization).

The most widely used cell immortalization methods are to over-express SV40 large T-antigen or human TERT gene. In addition to SV40-T-antigen and hTERT gene, other genes are also used for cell immortalization depending on cell types. The Epstein Barr Virus (EBV) genes (**EBNA1** and **EBNA2**) were reported used for immortalizing B and T lymphocytes, the HPV16 virus' **E6/E7** genes for keratinocytes, the Adenovirus type 5's **E1A** gene for primary rodent cells, the human HOX genes for various hematopoietic cells, including macrophages, hematopoietic progenitor cells, and myeloid progenitor cells, the human **CDK4** for human bronchial cells and myogenic cells, the human **KRas V12 mutant**, **cMyc** for a wide variety of cells and so on.

GenTarget provides the premade over-expression lentivirus products for those cell immortalization genes. Each gene was expressed under an enhanced **EF1a** promoter which is active in almost all cell types and less likely to be silenced during long-term culture. Each Lentivirus is featured with an antibiotic-fluorescent fusion dual marker, **GFP**-puromycin or **RFP**-Blasticidin. (see **vector map scheme** below).



VSV-G pseudotyped lentivirus are generated in 293T cell, and provided as 200 ul aliquots in two formats:

- 1) in DMEM medium containing 10% and 10x Polybrene (60 ug/ml) at titer of  $1 \times 10^7$  IFU/ml;
- 2) in PBS solution at titer of  $1 \times 10^8$  IFU/ml, for usage in serum-free cell culture;

For general questions about our ready-to-use particles, please see **FAQ for pre-made lentiviral particles** (.pdf) on our website.  
(<http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf>).



## Key features:

1. Each lentiviral particles contain an **antibiotic-fluorescent** fusion dual marker, used for selecting the transduced cells or generating stable cell lines by antibiotics selection or via fluorescent cell sorting.
2. The enhance **EF1a promoter** is active in all cell types and do not be silenced during long-term culture.
3. The lentivirus are ready and easy to use, simply add 50ul into one well culture in 24-well plate. No need any other reagents at application.

## Transduction Protocols:

### 1) Transduction Protocol for Adhesive cells :

**Note:** Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50  $\mu$ l of virus into each well of 24-well-plate where cell density is 50% to 75%. After 48 to 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For cell selection, pass cells into medium containing corresponding antibiotic or perform fluorescence cell sorting.

#### **Day 0:**

Seed cells in complete medium at the appropriate density and incubate overnight.

**Note:** at the time of transduction, cells should be 50%-75% confluent. For example, seed HeLa cells at  $0.5 \times 10^5$ /ml x 0.5ml in a well of a 24-well plate.

#### **Day 1:**

- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI.
- Return cells to 37°C, CO<sub>2</sub> incubator.

**Note:** Try to avoid freezing and thawing. If you do not use all of the virus at one time, you may re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

#### **Day 3:**

Depends on cell types, at 48~72hr post transduction, check the transduction rate by fluorescence microscopy (if applicable) or calculate the exact transduction rate by flow cytometry (FACS or Guava). Cells are ready for antibiotic selection.



## Day 3 / +:

Select transduced cells via antibiotic resistance. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines).

## **2) Transduction Protocol for Suspension Cells:**

Grow cells in complete suspension culture medium; use a shaking flask in a CO<sup>2</sup> incubator if necessary.

Measure cell density. When density has reached  $\sim 3 \times 10^6$  cells/ml, measured viability should be  $> 90\%$ . Dilute cells into  $1 \times 10^6$  cell/ml in complete medium.

### Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentivirus into the diluted cells at a ratio of: 50 to 100  $\mu$ l virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO<sub>2</sub> incubator.

### Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO<sub>2</sub> incubator.

### Day 3:

At 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells or select the cell by antibiotic killing, and maintain antibiotic selection to generate a stable cell line.

### **Note: Filter wavelength settings:**

**GFP filter:**  $\sim$ Ex450-490;  $\sim$ Em525; **RFP filter:**  $\sim$ Ex545;  $\sim$ Em620;

## **Safety Precaution:**

GenTarget lentiviral particles adapt must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the premade lentivirus is replication incompetent. However, please use extra caution when using lentiviral particles. Use the lentiviral particles in Bio-safety II cabinet. Wear glove all the time when handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.



## References:

1. Experimental Cell Research Volume 201, Issue 2, August 1992: 417-435
2. Genome Res. 2008 122 (3-4): 263-72.
3. Proc Natl Acad Sci U S A. 2003 Sep 16; 100(19): 10989-10994.
4. Semin Cancer Biol. 2001 Dec;11(6):423-34.
5. July 5, 2002. The Journal of Biological Chemistry 277, 24709-24716.
6. Mol. Cell. Biol. March 1988 vol. 8 no. 3 1036-1044
7. Methods in Enzymology Volume 439, 2008, Pages 1-13
8. Oncogene 2000 19, 608-616
9. Cancer Res. 2004 Dec 15;64(24):9027-34.
10. Cell. Volume 82, Issue 1, p29-36, 14 July 1995
11. Cancer Res 2005; 65: (6). March 15, 2005

## Warranty:

**This product is for research use only.** It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.

## Attachment: GenTarget's Pre-made lentivirus Product List:

<b>Product Category</b>	<b>Product Description (Click Category name to see product's pages)</b>
<a href="#">Gene Expression</a>	Premade lentivirus expressing <b>hundred of human and mouse ORFs</b> with RFP-Blastididin fusion dual markers.
<a href="#">Fluorescent Markers</a>	Premade Lentivirus for <b>GFP/ CFP/ YFP/ RFP</b> expression.
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">Pathway Specific Reporter</a>	Premade Lentivirus expressed a fluorescent marker or a luminescence marker under a pathway specific promoter.
<a href="#">Cell-Specific Reporter</a>	Premade Lentivirus expressed a fluorescent marker or a luminescence marker under a cell type specific promoter.
<a href="#">Luciferase Expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla</b> with different antibiotic selection markers.
<a href="#">Cell Immortalization</a>	Premade Lentivirus for Cell Immortalization: SV40 T-antigen; hTERT; EBV; HPV16-E6/E7; Adeno-E1A and more.
<a href="#">CRE Recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different fluorescent and antibiotic markers.
<a href="#">LoxP</a>	Premade lentivirus expressing " <b>LoxP-GFP-Stop-LoxP-RFP</b> "



<a href="#">ColorSwitch</a>	cassette, used to monitor the CRE recombination event in vivo.
<a href="#">TetR inducible expression repressor</a>	Premade lentivirus expressing <b>TetR (tetracycline regulator)</b> protein, the repressor protein for the inducible expression system.
<a href="#">CRISPR / CAS9</a>	Premade Lentivirus express CAS9 enzyme for genomic gene editing.
<a href="#">iPS factors</a>	Premade lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FGF4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">Cell Organelle imaging</a>	Premade lentivirus for cell organelle imaging. The fluorescent marker <b>GFP/RFP/CFP</b> was sub-cellular localized in different cell organelle for living cell imaging.
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>-galactosidase (lacZ)</b> with different selection markers
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific anti-miRNA cassette.
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">Pre-made shRNA lentivirus</a>	Premade shRNA lentivirus for knockdown a specific genes ( <b>P53, LacZ, Luciferase</b> and more).
<a href="#">microRNA and anti-microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme expression</a>	Ready-to-use lentivirus, expressing a <b>specific enzymes</b> with different selection markers.